Claims

1. (previously presented) A test strip for determining presence and/or amount of an analyte in a liquid sample comprising:

a mobilization zone;

a mobile or mobilizable detectable tracer in the mobilization zone;

a sample application area;

a primary capture area comprising a first immobilized binding partner having a binding affinity for the analyte and a binding affinity for the detectable tracer; and

a secondary capture area comprising a second immobilized binding partner having a binding affinity for the detectable tracer,

wherein the sample application area, mobilization zone, primary capture area and secondary capture area are in a path of liquid flow along a bibulous substrate from the sample application area distally through the mobilization zone to the primary capture area and then to the secondary capture area, wherein the detectable tracer is present on the test strip in a position that a distal flow of analyte reaches the primary capture area before a distal flow of tracer reaches the primary capture area such that subsequent binding of detectable tracer to first immobilized binding partner is inhibited and unbound detectable tracer continues along the path of flow distally to the second immobilized binding partner to provide a signal from the secondary capture area that indicates the presence of the analyte in the liquid sample.

2. (previously presented) The test strip of claim 1, wherein the detectable tracer is positioned within the test strip in a position that the distal flow of tracer reaches the primary capture area after the distal flow of analyte reaches the primary capture area.

3. (previously presented) The test strip of claim 1, wherein the detectable tracer is heavier than the analyte and therefore migrates through the test strip at a rate slower than a rate at which the analyte in ~~a~~ the liquid sample migrates through the test strip.

4. (previously presented) The test strip of claim 3, wherein the detectable tracer is selected to interact with the test strip to slow migration of the tracer relative to migration of the

analyte.

5. (previously presented) The test strip of claim 2, wherein the test strip is a bibulous porous strip and the detectable tracer is larger than the analyte and therefore migrates along the path of liquid flow through the bibulous porous strip more slowly than the analyte migrates along the path of liquid flow.

6. (previously presented) The test strip of claim 5, wherein the detectable tracer is positioned beneath the surface of the test strip on which the liquid sample is placed such that the detectable tracer migrates through the test strip to the primary capture area more slowly than analyte in the liquid sample.

7. (previously presented) The test strip of claim 4, wherein the tracer is selected based on its polarity or charge to provide specific migration characteristics that retard migration of the tracer relative to migration of the analyte.

8. (previously presented) The test strip of claim 7, wherein the test strip is pretreated with at least one reagent to enhance the sensitivity of the assay device by delaying migration of the tracer relative to the analyte.

9. (previously presented) The test strip of claim 8, wherein the at least one reagent is selected from the group consisting of sucrose, mannitol, glycerol, polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), and mixtures thereof.

10. (previously presented) The test strip of claim 1, wherein the first and second immobilized binding partners are antibodies, antigens, haptens, lectins or receptors.

11. (previously presented) The test strip of claim 1, wherein the first and second immobilized binding partners for the analyte are identical.

12. (previously presented) The test strip of claim 1, wherein the first and second immobilized binding partners are each antibodies, and the detectable tracer comprises an analyte analog, and the first binding partner is an antibody having a greater affinity for the analyte than the analyte analog.

13. (previously presented) The test strip of claim 1, wherein the detectable tracer comprises an analyte analog.

14. (previously presented) The test strip of claim 1, wherein the detectable tracer comprises a visually detectable label covalently attached to analyte or an analyte analog.

15. (previously presented) The test strip of claim 1, wherein the detectable tracer comprises a detectable tracer for an analyte selected from the group consisting of an antigen of an infectious disease, an antigen to an antibody of an infections disease, a hormone, a growth factor, a therapeutic drug, a drug of abuse, a product of the metabolism of a drug of abuse, and a hapten.

16. (previously presented) The test strip of claim 15, wherein the detectable tracer comprises a detectable tracer for an analyte comprising an antibody selected from the group consisting of an antibody to Human Immunodeficiency Virus (HIV), an antibody to Human T-Cell Lymphotropic Virus (HTLV), an antibody to *Helicobacter pylori*, an antibody to hepatitis, an antibody to measles, an antibody to mumps, and an antibody to rubella.

17. (previously presented) The test strip of claim 15, wherein the detectable tracer comprises a detectable tracer for an analyte comprising a therapeutic drug or drug of abuse or products of the metabolism of a drug of abuse, wherein the analyte is selected from the group consisting of tetrahydrocannabinol, nicotine, cotinine, ethanol, theophylline, phenytoin, acetaminophen, lithium, diazepam, nortryptyline, secobarbital, and phenobarbitol, methamphetamine and fragments, mimetics, and analogs or derivatives thereof.

18. (previously presented) The test strip of claim 17, wherein the detectable tracer

comprises a detectable tracer conjugate for an analyte that is a product of metabolism of a drug of abuse, and the product of metabolism comprises cotinine.

19. (previously presented) The test strip of claim 15, wherein the detectable tracer comprises a detectable tracer conjugate for an analyte comprising a hormone, and the hormone is selected from the group consisting of testosterone, estradiol, estriol, 17-hydroxyprogesterone, progesterone, thyroxine, thyroid stimulating hormone, follicle stimulating hormone, and luteinizing hormone, and fragments, mimetics, analogs or derivatives thereof.

20. (previously presented) The test strip of claim 1, wherein the quantity of detectable tracer binding to the secondary capture area, and by correlation the amount of the analyte in the liquid sample, is indicated by intensity of a detection signal of the detectable tracer in the secondary capture area.

21. (previously presented) The test strip of claim 1, wherein the secondary binding area of the test strip is divided into at least two discrete and non-overlapping bands, wherein the quantity of tracer binding to the secondary capture area, and by correlation the amount of the analyte in a tested sample, is indicated by the number of bands to which the tracer molecule binds.

Claims 22-30 (canceled)

31. (previously presented) A method for detecting and/or quantitating an analyte in a liquid sample, comprising:

contacting the liquid sample with the sample application area of the test strip of claim 1, and allowing the liquid sample to mobilize the tracer such that the distal flow of tracer migrates with the liquid sample, but reaches the primary capture zone after distal flow of analyte in the liquid sample;

wherein the distal flow of analyte that reaches the primary capture zone occupies first immobilized binding partner such that subsequent binding of the detectable tracer to the first immobilized binding partner is inhibited, whereby unbound detectable tracer continues along the path of flow distally to bind to the second immobilized binding partner and provide a signal from the

secondary capture area that indicates the presence of the analyte in the liquid sample; and

detecting a signal from the secondary capture area to detect and/or quantitate the analyte.

Claims 32-34 (canceled)

35. (previously presented) The method of claim 31, further comprising quantifying an amount of analyte in the liquid sample, wherein the amount of analyte in the liquid sample determines an intensity of the signal from the tracer in the secondary capture area.

36. (previously presented) The method of claim 31, wherein the liquid sample migrates along the test strip in the path of liquid flow by capillary action.

37. (original) The method of claim 31, wherein the analyte has a molecular weight of about 100 – 1,000 Daltons.

38. (original) The method of claim 31, wherein the analyte has a molecular weight of greater than 1,000 Daltons.

Claim 39 (canceled)

40. (previously presented) The method of claim 31, wherein the liquid sample is selected from the group consisting of urine, blood, tears, sweat and saliva.

41. (previously presented) The method of claim 40, wherein the liquid sample is saliva.

42. (previously presented) The method of claim 41, wherein the saliva is combined with a bile acid or bile salt in a concentration that reduces occurrence of false positives in the immunoassay.

43. (previously presented) The method of claim 42, wherein the bile acid or bile salt ranges in concentration from about 0.1 weight percent to about 1.0 weight percent of the saliva/bile salt or

saliva/bile acid combination.

44. (previously presented) The method of claim 43, further comprising contacting a chelator of divalent cations with the saliva sample.

45. (previously presented) A test kit for the detection and/or the determination of an analyte in a sample comprising:

(a) the test strip of claim 1; and

(b) instructions for using the test strip such that the flow of analyte in the liquid sample reaches the primary capture zone before the flow of tracer.

Claim 46 (canceled)

47. (previously presented) The method of claim 31, wherein the detectable tracer is contained beneath an external surface of the test strip, and the liquid sample is applied to the external surface of the test strip, such that the flow of tracer migrates at a slower rate along the path of flow than flow of analyte in the liquid sample migrates along the path of flow toward the primary capture area.

Claim 48 (canceled)

49. (previously presented) The method of claim 31, wherein the detectable tracer interacts with the test strip to slow its flow along the path of liquid flow more than flow of analyte in the liquid sample is slowed such that any analyte in the liquid sample reaches the primary capture zone ahead of the detectable tracer.

Claims 50-74 (canceled)